



Manual

ChlamType-23S AS-4 Kit

For the identification of ***Chlamydia* species** and other ***Chlamydiales***

23S based

Array Hybridisation Kit

Kit order number: 246500096

96 reactions (**ArrayStrip** format)

For Research Use Only. Not Intended for Use in Clinical Diagnostics.

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BACKGROUND

The ALERE **ChlamType-23S AS-4 Kit** allows the rapid, economic and standardised DNA-based detection of all currently recognised *Chlamydia* species and other *Chlamydiales*. Extracted DNA from different possible sources is exponentially amplified in a specific PCR with 5'-Biotin-labelled primers. The resulting biotin-labelled dsDNA is transferred and hybridised to DNA microarrays with 57 oligonucleotide probes for different genetic markers plus essential controls. All of them are spotted three times. Based on a digital image of the arrays, spot recognition is performed automatically and results are provided as a html-file providing species identification.

Two different methods for species discrimination were used:

1. **Pattern Match:** Comparison of an obtained hybridisation pattern with a set of 94 different theoretical and 14 experimental reference experiment patterns and subsequent assignment resulting in the best three matches. (See page 35).
2. **Chlamydia Species Assignment after Signal Interpretation:** Discrimination of *Chlamydia* species and other *Chlamydiales* using single markers and marker combinations.
 - **Controls:**
 - staining controls using biotinylated control spots
 - negative control spots
 - internal amplification and hybridisation controls:
 - DNA template for internal amplification reaction (target EGFP)
 - Marker indicating *E. coli* DNA contamination in the amplification reaction
 - **Family and Genus markers**
 - **Chlamydia species:**
 - *C. abortus*
 - *C. caviae*
 - *C. felis*
 - *C. psittaci*

- *C. pecorum*
- *C. pneumonia*
- *C. suis*
- *C. muridarum*
- *C. trachomatis*
- *C. avium*
- *C. gallinacea*

- **Other Chlamydiales:**

- | | |
|---------------------------------------|------------------------------------|
| - <i>Simkania</i> | - <i>Parachlamydia</i> |
| - <i>Waddlia</i> | <i>acanthamoebae</i> |
| - <i>Protochlamydia amoebophila</i> | - <i>Criblamydia sequanensis</i> |
| - <i>Protochlamydia aegleriophila</i> | - Chlamydiales <i>Xenoturbella</i> |
| - <i>Neochlamydia artmannellae</i> | - <i>Estrella lausannensis</i> |

GENERAL INSTRUCTIONS FOR USE

Intended Use

For Research Use Only. Not for Use in Diagnostic Procedures.

This assay allows the characterisation of amplified and labelled DNA originating from samples containing *Chlamydia* species and other *Chlamydiales*. The assay is made for research use and epidemiological applications. It should not be applied to other targets than *Chlamydia* and *Chlamydiales*.

Specifications

Upon receipt, the assay components need to be stored at different temperatures as specified in the package insert. The assay has to be performed at an ambient temperature of 18 °C to 28 °C.

Technical Support

If you require any further information on this product please contact:

Email: cct.home@clondiag.com

Phone: +49 (0) 36 41 3111-0

Fax: + 49 (0) 36 41 3111-120

For up-to-date information regarding the kit, please visit our website

<http://www.alere-technologies.com>

Safety Precautions

- The assay is intended for use by personnel trained in microbiological and molecular methods. Preparation of DNA from *Chlamydia* requires expertise in microbiology/molecular biology, and the local regulations for handling pathogenic microorganisms (biosafety level 2, avian *C. psittaci* biosafety level 3) are to be obeyed.
- Extracted chlamydial DNA from the different sources may be processed without further biosafety precautions, although contamination needs to be ruled out.
- Always wear protective clothing as required for laboratory work according to your specific regulations on laboratory safety.

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, the enclosed reagents do not require a Material Safety Data Sheet (MSDS). They do not contain more than 1 % of a component classified as hazardous and do not contain more than 0.1 % of a component classified as carcinogenic. Therefore, MSDS are not provided. Nevertheless, the buffers may cause irritation if they come into contact with eyes or skin, and may cause harm if swallowed. The regular precautions associated with laboratory work should be obeyed (e.g., wear protective goggles, gloves and lab coat and avoid contact with the reagents). If liquids have been spilled, clean with a disinfectant and/or laboratory detergent and water.

Alere assumes no liability for damage resulting from the handling of or contact with these products. If you have any questions, please contact our Technical Support (see above).

Shipping Precautions

RID/ADR: *Kein Gefahrgut* / No dangerous goods

IMDG: No dangerous goods

IATA: No dangerous goods

REAGENTS AND DEVICES

Kit Components, Storage and Stability

All reagents are provided in surplus (see below). If necessary, all components may be ordered separately. Please refer to the catalogue reference numbers (Cat#) at page 32 of this manual. For pricing please contact your local representative or our customer service, respectively.

The expiry date can be found on each bottle and on the outer packaging. All components have been stability tested for short term shipment (< 1 week) at ambient temperature (< 37 °C). The assay components with limited stability are D1 and C3. The other kit components have been proven to be stable for six months after expiry.

Suggested Reagents for DNA Labelling and Amplification (Recommended but not included.)

- **Taq Polymerase S** (5 U/μl), **10x Amplification buffer** and **25 mM MgCl₂**: Genaxxon Taq Polymerase S (high specificity) , Genaxxon BioScience GmbH (<http://www.genaxxon.de>) Cat# M3001.0500
- **dNTPs, 10 mM each**: Genaxxon PCR dNTP-Mix (Na salt), Genaxxon BioScience GmbH (<http://www.genaxxon.de>) Cat# M3016.1010
- **Double distilled (dd) water**
- **INTYPE IC-DNA** QIAGEN Leipzig GmbH, Cat# 05-902/1 (<http://www.lab-leipzig.de>) as EGFP template (2 x 10⁵ copies/μl)

Suggested Reagents for DNA Labelling and Amplification (Included)

- **23S_Foreward Primer** (100 μM): Forward primer, Metabion, 5'-ATTGAMAGGCGAWGAAGGA-3'
 - **23S_Reverse Primer_BIOTIN** (100 μM): Reverse primer, Metabion, 5'-Bio-GCYTACTAAGATGTTTCAGTTC-3'
- Target gene: *Chlamydia* 23S rRNA, Amplicon: 171 bp
- **EGFP_Foreward Primer** (10 μM): Forward primer (for internal amplification and hybridisation control), Metabion, 5'-CAGCCACAACGTCTATATCATG-3'
 - **EGFP_Reverse Primer_BIOTIN** (10 μM): Reverse primer, (for internal amplification and hybridisation control) Metabion, 5'-Bio-CTTGACAGCTCGTCCATGC-3'

Target gene: Internal control, Amplicon: 276 bp

Suggested Reagents for Hybridisation and Detection (Included)

- ArrayStrips (12 x 8 samples),
Protected against light and sealed under inert gas. Store at 18 °C to 28 °C. To be used within two weeks after opening. Close the unused wells with caps to protect them against humidity and dust and store them in the dark. *Avoid any touching or scratching of the microarray surface at the bottom of the well. Do not store or handle unused wells at an air humidity of more than 60 % since this may irreversibly corrode the spots.*
- StripCaps (24 strips)
- C1: Hybridisation Buffer
Store at 18-28 °C, protect against sunlight. Surplus: 200 %.
- C2: Washing Buffer 1
Store at 18 °C - 28 °C, protect against direct sunlight. Surplus: 200 %.
- C3: HRP Conjugate 100 x
Store at 2-8 °C, protect against direct sunlight. Surplus: 100 %.
- C4: Conjugate Buffer
Store at 18 °C to 28 °C, protect against direct sunlight. Surplus: 200 %.
- C5: Washing Buffer 2
Store at 18 °C to 28 °C, protect against direct sunlight. Surplus: 500 %.
- D1: Horseradish Peroxidase Substrate
Store at 2-8 °C, protect against direct sunlight. Surplus: 50 %.

Instrumentation & Software

- ArrayMate Reader (to be ordered separately, for details see below)
The ArrayStrip based **ChlamType-23S AS-4 Kit** can be processed on the ArrayMate reader only. Alternative devices ATR01/03 are not suitable for reading ArrayStrips. If you have questions, please contact us.
- Iconoclust software (provided with the reader)
- Test-specific software plug-in (can be downloaded from Alere Technologies website, check periodically for updates, for details see below). Specific information, such as spot names, marker names, location of the spots on the array, and size of the image taken by the reader's camera, is delivered with the reader or can be downloaded from our website. These test-specific plug-ins will be updated occasionally. Please check the NEWS section of our website www.alere-technologies.com.
- Support is available via cct.home@clondiag.com.

Components Required but Not Provided

- DNA preparation kits:
The assay has been tested using the DNeasy Blood & Tissue Kit by Qiagen (Cat# 69504) and the High Pure PCR Template Preparation Kit from Roche (Cat# 11796828001). Kits from other suppliers can be used if validated for the assay.

Please note: The DNA specimen needs to be sufficient in quality for PCR reactions.

- Equipment for DNA isolation, e.g. pipettes, centrifuge, thermoshaker, or automated device
- Photometer (OD 260 nm) for measuring the DNA concentration
- Equipment for non-denaturing agarose DNA gel electrophoresis for quality control of DNA
- Thermocycler for PCR
- Thermoshaker for hybridisation

Please note: We strongly recommend the BioShake iQ by QInstruments

(<http://www.qinstruments.com/>) equipped with a customised heating block

designed to fit ArrayStrips. Alternatively, you may use Eppendorf's Thermomixer

Comfort equipped with a heating block for microtiter plates.

- Pipettes: Suitable for 1 µl-5 µl volumes, 90 µl, 100 µl, 200 µl, and 1000 µl
- Multichannel pipettes for 100-200 µl
- Sterile reaction vials suitable for PCR (VWR Cat# 732-0098).
- Ultrapure (PCR grade) water
- Pasteur pipettes (VWR Cat# 612-2856).

AMPLIFICATION AND INTERNAL BIOTIN LABELLING

We provide all primers. All other recommended components should be purchased from a supplier of your choice.

EGFP_Forward Primer, EGFP_Reverse Primer_BIOTIN and INTYPE IC-DNA are necessary if you use the Internal Amplification and Hybridisation Control EGFP. If you don't use the control, please ignore the error message on the report page 1.

If you use field samples or other samples with very low initial DNA concentrations, please make sure to run the PCR amplification reactions in two single runs (instead of running them as duplex PCR), combine the 2 PCR reactions afterwards, and hybridize them against one and the same microarray.

We recommend including external amplification controls that contain DNA of *Chlamydia* reference strains as positive control and in addition, double distilled water as negative control.

Prepare a master mix by combining the following components per sample:

23s_PCR	Components	Volume in μ l	Final concentration
1. water	double distilled water	13.8	
2. buffer	10x Genaxxon Amplification Buffer	2.0	1x
3. $MgCl_2$	25 mM $MgCl_2$ Genaxxon	1.2	1.5 mM
4. dNTP Mix	dNTPs, 10 mM each Genaxxon	0.4	0.2 mM
5. Primer P1	23S_Forward Primer (100 μ M)	0.1	500 nM
6. Primer P2	23S_Reverse Primer_BIOTIN (100 μ M)	0.1	500 nM
7. Primer P3	EGFP_Forward Primer (10 μ M)*	0.1	50 nM
8. Primer P4	EGFP_Reverse Primer_BIOTIN (10 μ M)*	0.1	50 nM
9. Polymerase	Genaxxon Taq Polymerase S (high specificity), 5U/ μ l	0.2	1U
10. EGFP-Template	INTYPE IC-DNA (2 x 10 ⁵ copies/ μ l)*	1.0	1x10 ⁴ copies/ μ l

*Replace with dd water if you don't use the control

- Add **1 μ l of *Chlamydia* DNA** to 19 μ l of the master mix. Do not forget to label the vial properly.
- Conduct amplification in a programmed thermocycler (e.g., Eppendorf Mastercycler gradient with heated lid) according to the following temperature-time profile:

Pre-heat cover/lid to 105 °C	
60 sec at 96 °C	
40 cycles with	30 sec at 94 °C
	30 sec at 60 °C
	30 sec at 72 °C
240 sec at 72 °C	
Cool down to 4 °C, hold	

- The samples can be stored at – 20° C until use.

Please note: When using another thermocycler, minor adaptations to the program might be necessary. Proper validation involving reference samples and controls is indispensable before routine use of the assay.

Hybridisation

General Remarks for the Handling of Arrays

- *Never touch the array surface.*
- *Avoid complete drying of the array surface during processing.*
- *Do not allow the array to stay without liquid for more than two minutes.*
- *Never rinse the wells with distilled water after the hybridisation step, only use C2 Washing Buffer.*

Unused wells should remain capped during the whole procedure. The strips may be processed up to three times without loss of quality of properly capped unused arrays. Close all wells that will not be used with a cap and leave them there until use (for storage conditions after use: see section ‘Kit components, storage and stability/Hybridisation and Detection’).

Always label your array strips with a laboratory marker at the recommended position. **Never** label them on the bottom or across the data matrix barcode. This may cause errors.



Avoid contact of data matrix barcode with organic solvents. The ArrayMate needs the information encoded in the data matrix to perform the assay and the analysis afterwards.

Avoid touching the bottom of the microarray strip and keep it clean.

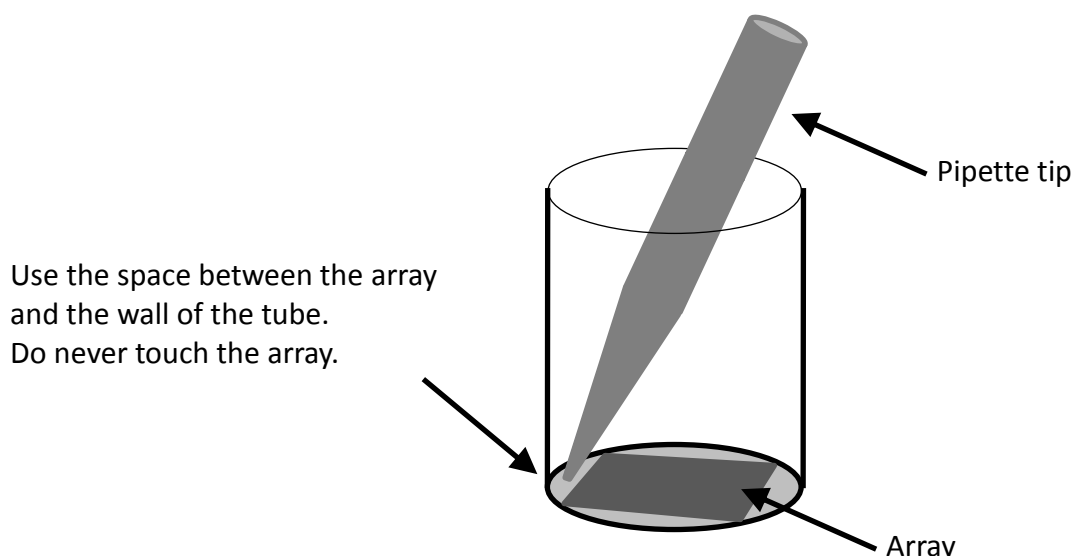
General Remarks for the Handling of Liquids

We recommend the use of a multichannel pipette and reagent reservoirs.

We strongly recommend that the liquid is removed by pipetting rather than inverting the strips and pouring the liquids out. Disposable fine-tipped soft Pasteur pipettes (such as VWR / Cat# 612-2856) are suited best. Always place the pipette tip at the space between the array and the wall of the reagent well. If you touch the array surface, probes may be scratched off, and this may cause errors.

Pasteur pipette, plastic, with a flexible tip:





General Remarks - The Substrate (Precipitating Dye) D1

An appropriate amount of D1 substrate (precipitating dye) should be transferred into an Eppendorf tube and taken out of the refrigerator when starting the procedure to allow it to pre-warm to room temperature (25 °C). Cold D1 may yield weak signals. D1 should be centrifuged prior to use to remove bubbles as well as possible precipitates (quick spin).

Triggered by peroxidase, in the case of positive reactions, the dye precipitates but it is not covalently bound. The precipitate can be dissolved by vigorous shaking. Thus the arrays must **not be shaken**, dropped or moved abruptly during the staining procedure or thereafter.

After completion of staining, remove and discard reagent D1 as completely as possible and scan immediately. The dye precipitate fades slowly in presence of liquids.

General Remarks - Thermoshakers

The correct temperature within the vessels is essential; therefore always use the appropriate equipment for heating. Because of the possibility of inhomogeneous temperature distribution within the heating block, as well as possible differences between displayed and actual temperatures, the use of different brands of thermoshakers might affect test performance. We tested the assay with a BioShake iQ by QInstruments (<http://www.qinstruments.com/>) equipped with a customised heating block designed to fit ArrayStrips, as well as with an Eppendorf Thermomixer Comfort (<http://www.eppendorf.com/>), equipped with a heating block for microtiter plates and Deepwell plates (Eppendorf, Cat# 5363000012). When using

other devices, some modifications to the protocol might be necessary. Before starting routine use, please test the protocol with a few known reference strains. The only difference between the protocols for QInstruments BioShake iQ and Eppendorf's Thermomixer Comfort with Microtiter Plate Adapter is the washing temperature after the hybridisation step.

Protocol for Quantifoil's BioShake iQ



BioShake iQ by QInstruments
equipped with a customised heating
block designed to fit ArrayStrips.

- Preparation of the hybridisation mixture (denaturation):
 - Pipette 98 μ l of C1 buffer into a 1.5 ml tube and add 2 μ l labelled duplex amplification product (or 2 x 1 μ l amplification product in case of two single runs), close the tube and mix gently.
 - Incubate the mixture at 95 °C for 5 min (in a water bath or in a thermoshaker).
 - Cool down the tube on ice for 2 min.
- Pre-washing the arrays:
 - Switch on the thermoshaker and let it pre-heat to 58 °C.
 - Remove the ArrayStrip(s) from the pouch.
 - Insert the ArrayStrip(s) into the white frame. Make sure the orientation is correct (data matrix barcode close to row (A) and the strips fit properly).
 - Pre-wash the array(s) with 200 μ l PCR-grade distilled water per well: pipette the water 4 times up and down at room temperature. Remove the water from the well.

- Transfer the denaturated amplification product (100 µl) to one well of the prepared strip.
- Incubate at 58 °C and 550 rpm for 60 min.

Meanwhile, login to the ArrayMate device and prepare your worklist (see ‘Data Analysis’ section, p. 18)

- Remove the liquid and add 200 µl C2 Washing Buffer. Incubate at 43 °C and 550 rpm for 10 min, remove and discard.
- Add another 200 µl C2 Washing Buffer. Incubate at 43 °C and 550 rpm for 10 min.

Meanwhile, prepare conjugate: For each experiment add 1 µl 100x HRP conjugate to 100 µl C4 Conjugation Buffer. This mixture is stable at room temperature for around one working day; C3 is delivered with a surplus of 100 %, and C4 with a surplus of 200 %. Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 µl	3.5 µl	7 µl	11 µl	16 µl	21 µl	32 µl	42 µl
C4	150 µl	350 µl	700 µl	1100 µl	1600 µl	2100 µl	3200 µl	4200 µl

- Remove the Washing Buffer, and add 100 µl diluted conjugate to each well, incubate at 30 °C and 550 rpm for 10 min.
- Remove the conjugate, add 200 µl C5 Washing Buffer. Pipette the buffer 4 times up and down at room temperature.
- Remove the Washing Buffer, add 100 µl of D1 substrate (precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C for 5 min but **do not shake!**
- Remove liquid completely.
- The bottom of the ArrayStrips (outside surface) may be cleaned carefully with wipes. Bubbles may be removed by removing and adding D1.
- Scan and process (see below).

Protocol for Eppendorf's Thermomixer Comfort with Microtiter Plate Adapter



Eppendorf Thermomixer with thermoblock for MTPs and deepwell plates.

- Preparation of the hybridisation mixture (denaturation):
 - Pipette 98 μ l of C1 buffer into a 1.5 ml tube and add 2 μ l labelled duplex amplification product (or 2 x 1 μ l amplification product in case of two single runs). Close the tube and mix gently.
 - Incubate the mixture at 95 °C for 5 min (in a water bath or in a thermocycler).
 - Cool down the tube on ice for 2 min.
- Pre-washing the arrays:
 - Switch on the thermoshaker and let it pre-heat to 58 °C.
 - Remove the ArrayStrip(s) from the pouch.
 - Insert the ArrayStrip(s) into the white frame. Make sure the orientation is correct (data matrix barcode close to row (A) and the strips fit properly).
 - Pre-wash the array(s) with 200 μ l PCR-grade distilled water per well: pipette the water 4 times up and down at room temperature. Remove the water from the well.
- Transfer the denaturated amplification product (100 μ l) to one well of the prepared strip.
- Incubate at 58 °C and 550 rpm for 60 min.

Meanwhile, login to the ArrayMate device and prepare your worklist (see 'Data Analysis' section p. 18)

- Remove the liquid and add 200 μ l C2 Washing Buffer. Incubate at 50 °C and 550 rpm for 10 min, remove and discard.

- Add another 200 μ l C2 Washing Buffer. Incubate at 50 °C and 550 rpm for 10 min.

Meanwhile, prepare conjugate: For each experiment add 1 μ l 100x HRP conjugate to 100 μ l C4 Conjugation Buffer. This mixture is stable at room temperature for around one working day; C3 is delivered with a surplus of 100 %, and C4 with a surplus of 200 %.

Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 μ l	3.5 μ l	7 μ l	11 μ l	16 μ l	21 μ l	32 μ l	42 μ l
C4	150 μ l	350 μ l	700 μ l	1100 μ l	1600 μ l	2100 μ l	3200 μ l	4200 μ l

- Remove the Washing Buffer, and add 100 μ l diluted conjugate to each well, incubate at 30 °C and 550 rpm for 10 min.
- Remove the conjugate, add 200 μ l C5 Washing Buffer. Pipette the buffer 4 times up and down at room temperature.
- Remove the Washing Buffer, add 100 μ l of D1 substrate (precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C for 5 min but **do not shake!**
- Remove liquid completely.
- The bottom of the ArrayStrips (outside surface) may be cleaned carefully with wipes. Bubbles may be removed by removing and adding D1.
- Scan and process (see below).

Data Analysis

Starting the ArrayMate Reader

We recommend launching the ArrayMate Reader after starting the hybridisation; this allows the worklist file to be imported prior to the beginning of manual operations.

Please note that this is a short instruction only. For more detailed information please refer to the ArrayMate User Manual.

- Switch on the ArrayMate (1st: Main switch on the rear below the electric cable plug, 2nd: Operating switch on the lower left corner of the front side).
- Switch on the screen (switch is on the right hand side below the screen).
- Log-in as **R&D User** (Research and Development User) for full access to test-specific software (default password: abcde). If you log-in as **User**, you will obtain raw values, but neither positives/negatives interpretation nor strain assignment. The **Administrator** log-in (default password: 12345) will allow the installation of a new assay specific plug-in, which can be downloaded at <http://alere-technologies.com>.
- The user interface will be loaded, the ArrayMate performs internal testing. This requires slightly less than a minute.
- Click **New Run** (left edge of the screen). A suggestion for a run name/folder name for the new run appears in the top line of the screen. You may modify or change the experiment name at your convenience.
- Type in your operator ID (**optional**).

Worklist

A **Worklist** file allows an identifier, such as a laboratory or sample number, to be linked to the respective array position on the ArrayStrip. For privacy reasons, arrays or the respective experiments should not be denoted with patient names. Worklists can be generated using spreadsheet software such as EXCEL (see below), but must be saved in the *.txt file format, which can be imported into the test-specific ArrayMate software. **Do not include special characters** (such as : ; ()[] / \ ä ü etc.).

- Create a list with at least three columns having headers written in the first line. The following headers are obligatory (in this order): position / sampleID / assayID (Table 1).
- Positions are consecutively numbered from 1 to a maximum of 96. Position 1 would correspond to A1, 8 to H1, 9 to A2 and 96 to H12 (Table 2). Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be entered into column A.
- Sample IDs are strain/sample/laboratory numbers, e.g. as exported from your LIMS (or designated in any different way). Patient names should not be used as sample IDs.
- The Assay ID allows the system to identify the current test and to correctly use information on layout, spot number and identity etc. The **ChlamType-23S AS-4 Kit** has the Assay ID: **10454**.

Please note: When entering assay IDs manually, make sure to enter the correct number as this could lead to errors or loss of data.

- You may add further columns and headers with notes and comments at your convenience. Information from these columns will neither appear on the result screen nor in the Test Report.
- We recommend the use of a printout of the worklist as a template for pipetting.
- Save the worklist as **tab separated *.txt** file on the memory stick provided together with the ArrayMate.
- To avoid confusion, make sure that worklists are named unambiguously, or that worklists from earlier experiments are deleted.
- You may use the software tool Worklist Generator to create a Worklist easily.

<http://alere-technologies.com/en/products/lab-solutions/software-tools/worklist-generator.html>

Table 1: Worklist example

Please note: Table head must be written exactly as shown.

position	SampleID	assayID	assayType
1	2014-12345	010454	23s
2	2014-12346	010454	23s
3	2014-12347	010454	23s
4	2014-12348	010454	23s
5	2014-12349	010454	23s
6	2014-12350	010454	23s
7	DC48_Simkania	010454	23s
8	<i>C. caviae</i>	010454	23s

Table 2: Positions in the 96 well format

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
H	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Data Acquisition in the ArrayMate Reader

- Insert your flash drive containing the worklist into any of the USB ports on the lower right-hand side of the ArrayMate.
- Press ; a folder selection dialogue will open.
- Select your worklist (path: 'My Computer/Removable Disk').
- Open your selected worklist by pressing **Enter** or **Open**.
- Press  (your imported worklist opens in a separate window). Proofread. If the new window is empty, or if it was the wrong worklist, repeat the import.
- Press **OK**; the worklist window will close.

- Leave the flash drive in the ArrayMate if you intend to export **ChlamType-23S AS-4 Kit** reports afterwards (Check the flash drive regularly for computer viruses and malware using an appropriate program.).
- Press **Next** (at the bottom right on the screen; reader is opening).
- Carefully insert the appropriate metallic adapter/frame into the ArrayMate. Do not apply strong force. Ensure proper fit, otherwise the images may be out of focus.
- Carefully insert the white frame with the array strips into the metallic adapter. Ensure the correct orientation (Position A1 in the frame next to the data matrix barcode on the adapter) and proper fit; otherwise the images may be out of focus.



ArrayStrip frame with inserted strips. Strips are inserted in accordance with the **Worklist**.

Please note: ArrayStrips must be clean. They should not contain any liquids during analysis. Data matrix codes must be clean. There must be no Array StripCaps on the wells to be analysed (however, unused wells should remain capped).

- Press **Next** (at the bottom right on the screen; reader closes, analysis program starts, it takes about 2-10 min, depending on the number of strips; the reader takes images and automatically analyses the data). The progress of the reading is indicated by the following symbols:

photographed:



in analysis:



ready:



- The reader indicates the end of the entire process with an acoustic signal (beep).
- Press **Next** (at the bottom right on the screen; reader is opening).
- Remove the white frame with the ArrayStrip(s).
- Press **Next** (at the bottom right on the screen; reader is closing).

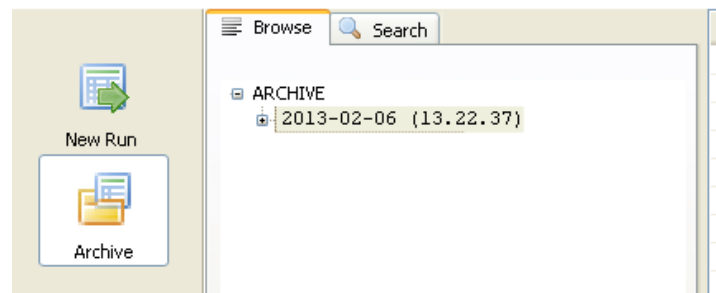
Results

On the left-hand side of the screen, there you will see a list showing all runs stored on the ArrayMate's hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

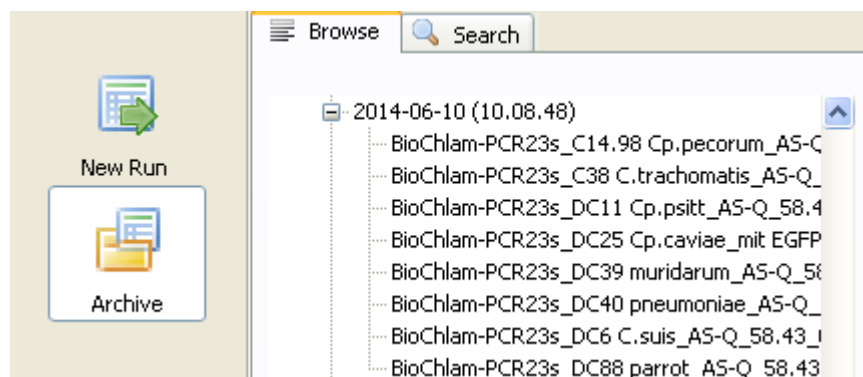
- Press **Archive** (left hand side) and activate the flag **Browse** (at the top left).

The runs are organised like folders in **Windows Explorer**, and named **by default** according to the date of acquisition.

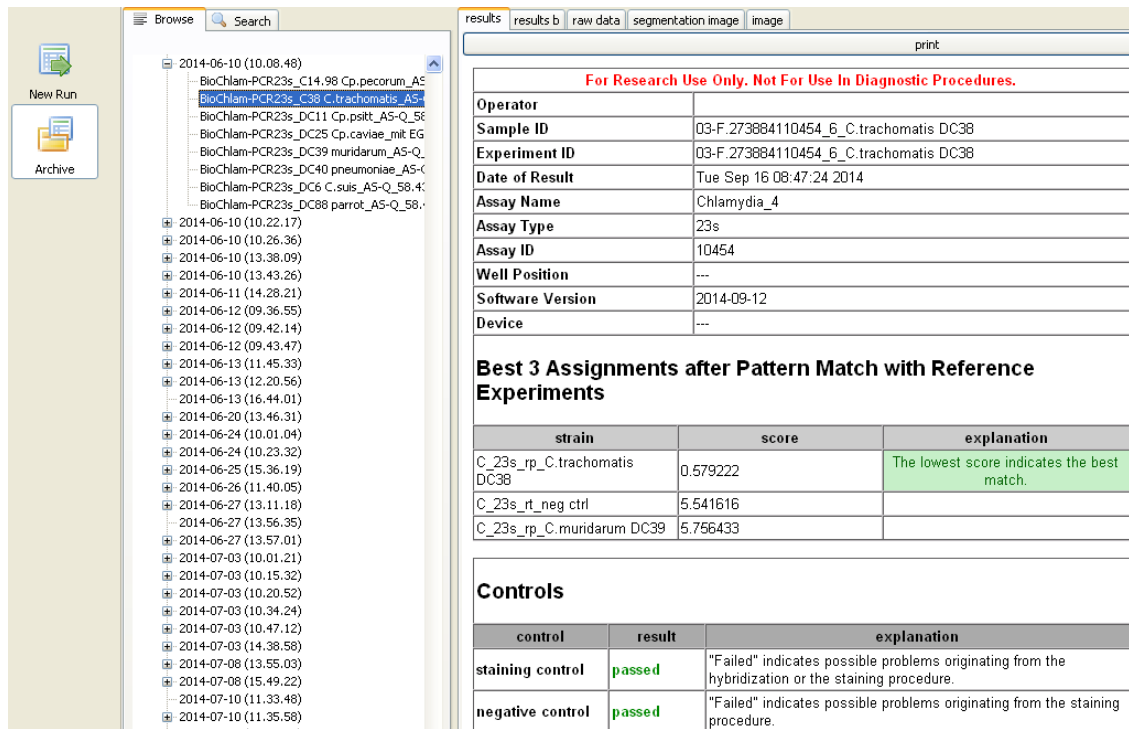
Example: There is one experiment run in this archive:



If you click on the plus symbol left to the run name, the folder opens and you will see a list of the individual arrays alphabetically ordered by Sample ID.



Click on a Sample ID and the **ChlamType-23S AS-4 Kit** test report (flag results) for this array is shown in the window on the right:



For Research Use Only. Not For Use In Diagnostic Procedures.

Operator	
Sample ID	03-F.273884110454_6_C.trachomatis DC38
Experiment ID	03-F.273884110454_6_C.trachomatis DC38
Date of Result	Tue Sep 16 08:47:24 2014
Assay Name	Chlamydia_4
Assay Type	23s
Assay ID	10454
Well Position	---
Software Version	2014-09-12
Device	---

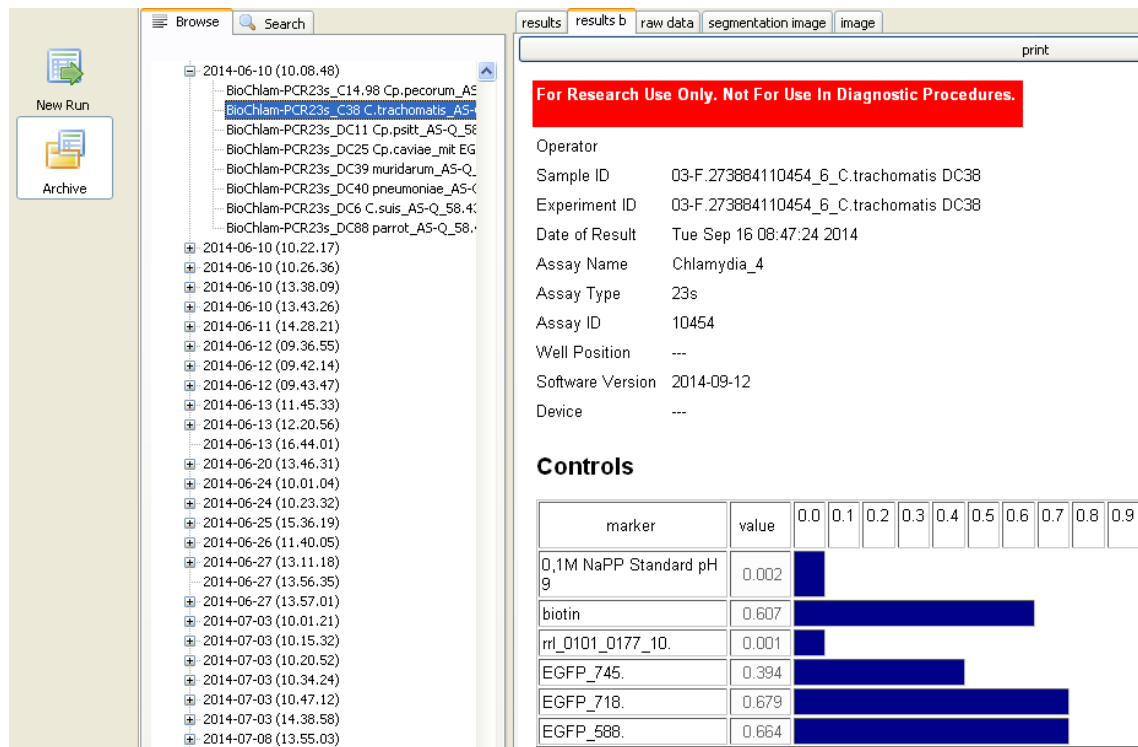
Best 3 Assignments after Pattern Match with Reference Experiments

strain	score	explanation
C_23s_rp_C.trachomatis DC38	0.579222	The lowest score indicates the best match.
C_23s_rt_neg ctrl	5.541616	
C_23s_rp_C.muridarum DC39	5.756433	

Controls

control	result	explanation
staining control	passed	"Failed" indicates possible problems originating from the hybridization or the staining procedure.
negative control	passed	"Failed" indicates possible problems originating from the staining procedure.

Click on flag resultsB and the Test Report B is shown in the window on the right:



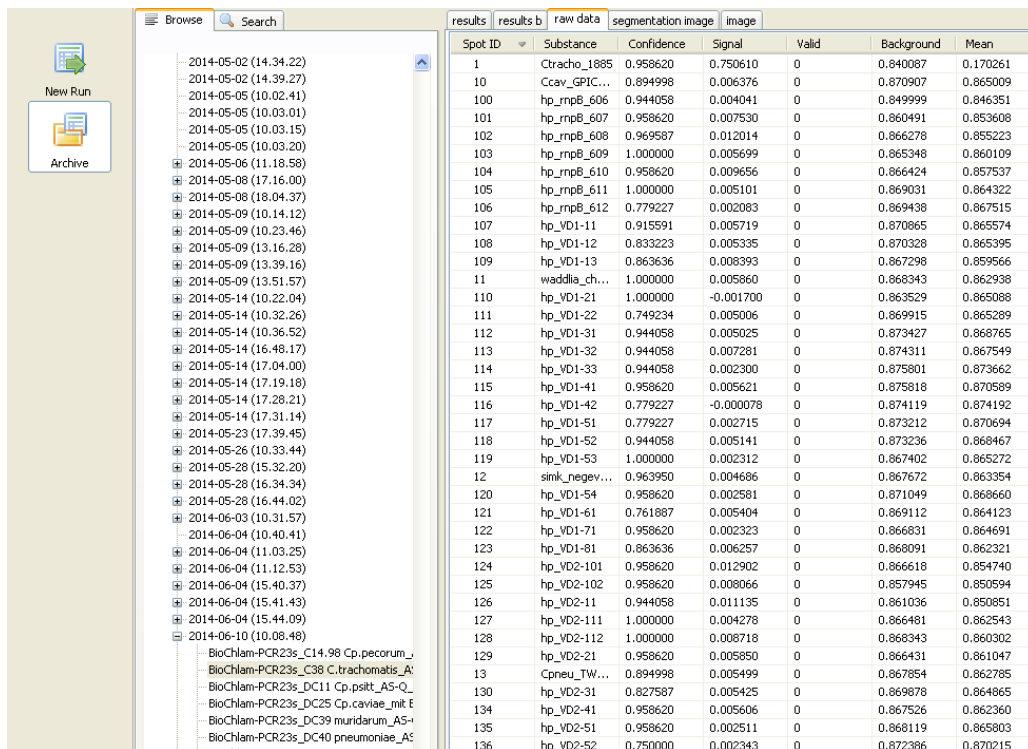
For Research Use Only. Not For Use In Diagnostic Procedures.

Operator	
Sample ID	03-F.273884110454_6_C.trachomatis DC38
Experiment ID	03-F.273884110454_6_C.trachomatis DC38
Date of Result	Tue Sep 16 08:47:24 2014
Assay Name	Chlamydia_4
Assay Type	23s
Assay ID	10454
Well Position	---
Software Version	2014-09-12
Device	---

Controls

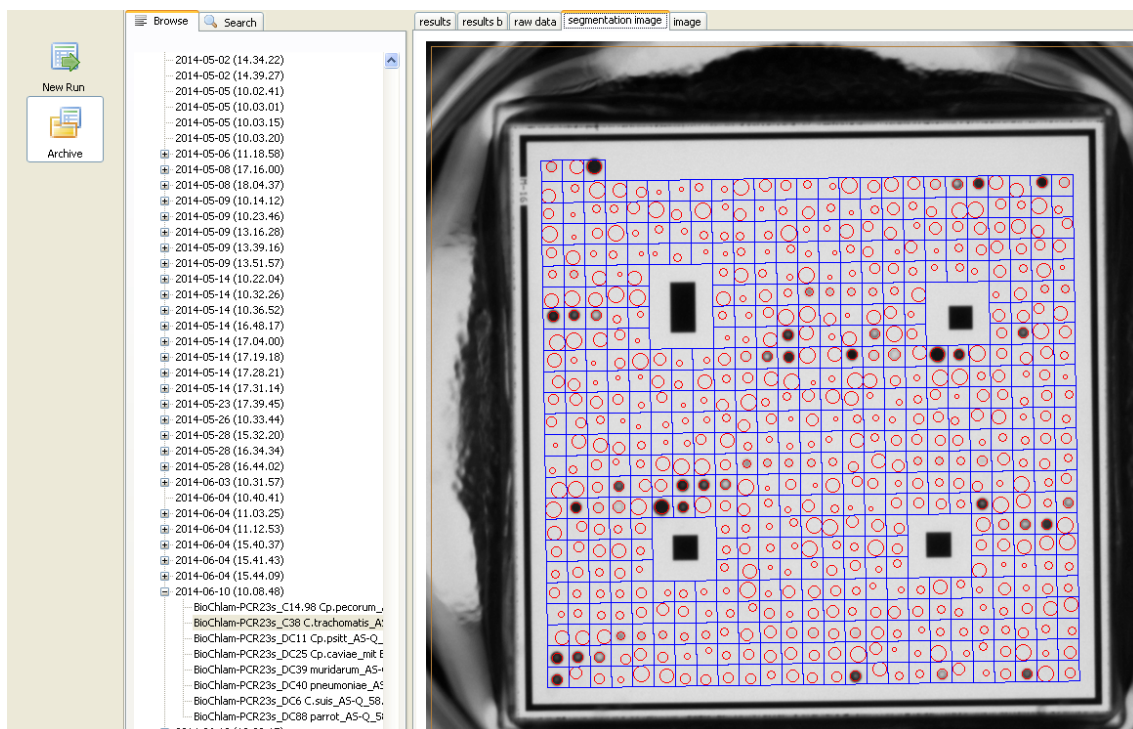
marker	value	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1M NaPP Standard pH 9	0.002										
biotin	0.607										
rrl_0101_0177_10.	0.001										
EGFP_745.	0.394										
EGFP_718.	0.679										
EGFP_588.	0.664										

Click on flag raw data and the raw data is shown in the window on the right:



Spot ID	Substance	Confidence	Signal	Valid	Background	Mean
1	Ctracho_1885	0.958620	0.750610	0	0.840087	0.170261
10	Ccav_GPIC...	0.894998	0.006376	0	0.870907	0.865009
100	hp_rmp8_606	0.944058	0.004041	0	0.849999	0.846351
101	hp_rmp8_607	0.958620	0.007530	0	0.860491	0.853608
102	hp_rmp8_608	0.969587	0.012014	0	0.866278	0.855223
103	hp_rmp8_609	1.000000	0.005699	0	0.865348	0.860109
104	hp_rmp8_610	0.958620	0.009656	0	0.866424	0.857537
105	hp_rmp8_611	1.000000	0.005101	0	0.869031	0.864322
106	hp_rmp8_612	0.779227	0.002083	0	0.869438	0.867515
107	hp_VD1-11	0.915591	0.005719	0	0.870865	0.865574
108	hp_VD1-12	0.833223	0.005335	0	0.870328	0.865395
109	hp_VD1-13	0.863636	0.008393	0	0.867298	0.859566
11	waddlia_ch...	1.000000	0.005860	0	0.868343	0.862938
110	hp_VD1-21	1.000000	-0.001700	0	0.863529	0.865088
111	hp_VD1-22	0.749234	0.005006	0	0.869915	0.865289
112	hp_VD1-31	0.944058	0.005025	0	0.873427	0.868765
113	hp_VD1-32	0.944058	0.007281	0	0.874311	0.867549
114	hp_VD1-33	0.944058	0.002300	0	0.875801	0.873662
115	hp_VD1-41	0.958620	0.005621	0	0.875818	0.870589
116	hp_VD1-42	0.779227	-0.000078	0	0.874119	0.874192
117	hp_VD1-51	0.779227	0.002715	0	0.873212	0.870694
118	hp_VD1-52	0.944058	0.005141	0	0.873236	0.868467
119	hp_VD1-53	1.000000	0.002312	0	0.867402	0.865272
12	simk_negev...	0.963950	0.004686	0	0.867672	0.863354
120	hp_VD1-54	0.958620	0.002581	0	0.871049	0.868660
121	hp_VD1-61	0.761887	0.005404	0	0.869112	0.864123
122	hp_VD1-71	0.958620	0.002323	0	0.866831	0.864691
123	hp_VD1-81	0.863636	0.006257	0	0.868091	0.862321
124	hp_VD2-101	0.958620	0.012902	0	0.866618	0.854740
125	hp_VD2-102	0.958620	0.008066	0	0.857945	0.850594
126	hp_VD2-11	0.944058	0.011135	0	0.861036	0.850851
127	hp_VD2-111	1.000000	0.004278	0	0.866481	0.862543
128	hp_VD2-112	1.000000	0.008718	0	0.868343	0.860302
129	hp_VD2-21	0.958620	0.005850	0	0.866431	0.861047
13	Cpneu_TW...	0.894998	0.005499	0	0.867854	0.862785
130	hp_VD2-31	0.827587	0.005425	0	0.869878	0.864865
134	hp_VD2-41	0.958620	0.005606	0	0.867526	0.862360
135	hp_VD2-51	0.958620	0.002511	0	0.868119	0.865803
136	hp_VD2-52	0.750000	0.002343	0	0.872386	0.870215

Click on flag segmentation image and the grid alignment accuracy is shown in the window on the right:



Click on flag image and the image file (*.bmp) is shown in the window on the right:



This image of the reference isolate *C. trachomatis* shows an example for a valid test without any dust particles or non-specific background.

The image file is automatically analysed by the ArrayMate software and a HTML report is provided that lists all markers that have been analysed.

Export of ChlamType-23S AS-4 Kit Test Report

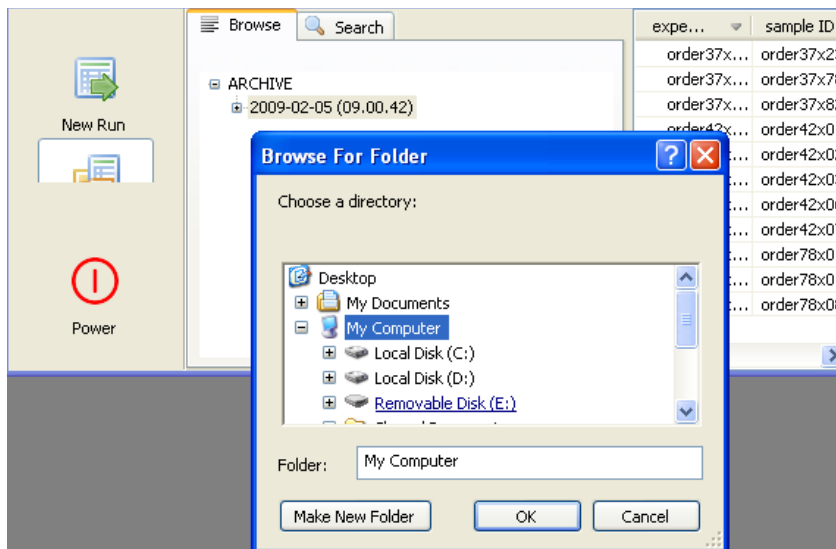
The generated result files in an html format will show information of all target genes. Possible invalid controls that might display in this report will be explained below (see Troubleshooting).

Other files that are generated and that can be exported include

- A *.txt file with the raw measurements,
- An image file (*.bmp) with the actual photo of the array,
- A second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled, and
- A XML (*.xml) files that contains the same information as the html result sheets for future export into databases etc.

Please note: Only complete runs can be exported. The export of individual ChlamType-23S AS-4 Kit Test Reports is not possible.

- Right-click on the selected run (a menu appears with the option **Export Run Reports**).
- Right-click on **Export Run Reports** (a file browser opens).



- Click **My Computer**, then **Removable Disk** and choose the folder where to save or click **Make New Folder** (on the bottom, a new folder icon appears).
- Rename the new folder (e.g. with the experiment name or date).
- Click **Ok** (data are exported into the new folder on your flash drive).
- Do **NOT** remove the flash drive as long as the hourglass symbol is visible.
- Switch off the device by clicking **Power** (at the bottom left on the screen): 
- Switch off the screen. There is no need to physically switch off the ArrayMate Reader.

TROUBLESHOOTING AND REPORT INTERPRETATION

In case of any troubleshooting make sure that reagents are within the recommended shelf-life and stored appropriately.

In case of problems we are always happy to provide support. Please e-mail to cct.home@clondiag.com and include a description of the problem, as well as the array images (*.bmp files) in question.

Two different methods for species discrimination were used:

1. **Pattern Match:** Comparison of an obtained hybridisation pattern with a set of 94 different theoretical and 14 experimental reference experiment patterns and subsequent assignment resulting in the best three matches. (See page 38). The pattern match algorithm provides an initial orientation and is not fully reliable in closely related patterns. Each theoretical reference pattern exists in 3 different variants (assuming high, medium and low stringency hybridisation conditions).
2. **Chlamydia Species Assignment after Signal Interpretation:** Discrimination of *Chlamydia* species and other *Chlamydiales* using single markers and marker combinations. This method might provide more accuracy, if the pattern match is not applicable. If both results are not in concordance, please stay with the result of the Chlamydia Species Assignment after Signal Interpretation.

Staining Control

A staining control is included to check whether possible problems originate from the hybridisation or the staining procedure. If the staining control has 'Failed' proceed as follows:

Horseradish peroxidase conjugate may have degraded during storage. Add 1 µl C3/C4 buffer to 9 µl D1 (substrate). If the solution turns green within 3-5 seconds, the horseradish peroxidase still has sufficient enzymatic activity.

Enzymatic reaction is inhibited by carryover of buffer C1. Ensure proper washing of the wells with C2 buffer to remove all C1 buffer prior to adding horseradish peroxidase conjugate.

Negative Control

'Failed' indicates possible problems originating from the staining procedure.

Marker rrl 0101 0177 10

Marker indicates *E. coli* DNA contamination. The presence of *E. coli* DNA (e.g. originating from the DNA polymerase production process) can reduce the signal intensity of other spots and hence yield false negative results. One can ignore this marker if the species identification is okay.

Plausibility Controls

The following error messages (Plausibility Controls) might appear in the report:

Internal Amplification and Hybridisation Control EGFP

At least one of three EGFP probes has to be positive. Otherwise an error message is shown:

Plausibility Controls		
control	result	explanation
Internal Amplification and Hybridisation Control	Failed	Absence indicates inhibition of DNA amplification! Please repeat the experiment!

A negative internal amplification and hybridisation control together with a negative species assignment might be due to potential inhibitors in your current DNA preparation.

A positive internal amplification and hybridisation control together with a negative species assignment might be due to a very low input of DNA copy numbers or fragmentation of the

DNA. Please repeat the amplification in two single tubes (not as duplex PCR) and hybridize it together in one and the same well.

A negative internal amplification and hybridisation control together with a positive species assignment might be due to a very high DNA copy number input.

The plausibility control can be ignored if you do not use INTYPE IC-DNA.

Family and Genus Control

If family or genus markers are too weak, negative or does not match, respectively, an error message appears:

Plausibility Controls		
control	result	explanation
Species Control	Failed	Weak or implausible species marker. Please repeat the experiment! Please note that other Chlamydiales will not generate family and genus signals (e.g. Simkania, Waddlia, Protochlamydia, Neochlamydia, Parachlamydia, Criblamydia, Chlamydiales or Estrella).

Mixed Infection Control

This error message appears if more than one species is found from chlamydia species assignment after signal interpretation, or if more than one genus marker (chlamydia and chlamydophila) is positive:

Plausibility Controls		
control	result	explanation
Mixed Infection Control	Positive	"Positive" indicates a mixed infection comprising two or more <i>Chlamydia</i> strains or DNA contamination.

Special case: If the species ***C. caviae* AND *C. felis*** or ***C. pecorum* AND *C. abortus*** give a mixed hybridisation pattern, a special mixed infection control statement appears:

Plausibility Controls		
control	result	explanation
Mixed Infection Control	Attention	If all probes for C. caviae AND C. felis give a signal, there are two possibilities of interpretation: i) presence of C. caviae only, while the C. felis signals are due to cross-hybridisation (if family marker pos_56_C is negative), ii) mixed infection of both species (in this case, family marker pos_56_C is positive; see page B).

Or:

Plausibility Controls		
control	result	explanation
Mixed Infection Control	Attention	If all probes for C. pecorum AND C. abortus give a signal, there are two possibilities of interpretation: i) presence of C. pecorum only, while the C. abortus signals are due to cross-hybridisation (if family marker pos_56_C is negative), ii) mixed infection of both species (in this case, family marker pos_56_C is positive; see page B).

Image Quality

In case of poor image quality, we recommend to re-check the labelled amplification product quantity and quality, first by loading the remaining PCR product on an agarose gel.

In order to determine whether any problems originated from the DNA preparation, conduct an experiment with control material. DNA from a number of *Chlamydia* spp. reference and clinical strains can be obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ; www.dsmz.de or other strain collections such as ATCC or Institute Pasteur). If the control experiment yields a valid result and a correct identification, there was probably an issue with DNA preparation. If the control experiment fails as well, an error in later steps or degradation of reagents from later steps is likely.

DNA Quality and RNA Contamination Control

The template DNA should be largely un-fragmented, as fragmentation reduces the amplification and labelling efficiency. For this reason DNA should not be prepared by using bead beaters, ultra-sonication or aggressive chemicals, such as those in alkaline lysis protocols. The present Assay has been validated using the QIAGEN DNeasy and the Roche High Pure kits. DNA should be free of any traces of ethanol, as ethanol inhibits the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 min at 70 °C) to evaporate the ethanol.

Physical Damage to the Array

Scratching the array surface with a pipette tip may damage array spots, which may lead to the impairment or absence of a valid signal. In this case, the respective marker will not be assigned as 'negative', but instead, the message 'none' appears next to the marker name.

Report Unavailable

If the ArrayMate indicates that no report is available for an array (or multiple arrays on one strip), please check that the strip has been positioned properly in the frame. Scratches or drops of condensed water might render the barcode identifier unreadable, so please wipe it carefully or try to identify the test manually. If no obvious reason for the fault can be detected, please contact the technical service.

ADDITIONAL INFORMATION

Warranty

Alere Technologies guarantees the performance as described in this user guide. The usage of the kit was successfully tested at ambient temperatures up to 37 °C. A guarantee is limited to ambient temperatures in the laboratory between 18 to 28 °C. Assay components comprise the arrays and their caps, the lysis enhancer, the reagents for DNA labelling and for detection of labelled DNA products on the array, the ArrayMate reader and its software. In case one of these components fails within the expiry date due to reasons other than misuse, contact Alere Technologies for replacement or refund. Terms and conditions apply.

If you have any problem or question, please contact the technical service.

Disclaimer

This system is for research use only.

We do not accept any liability for damages caused by misuse. That includes the use for diagnostic applications and for the guidance of therapy.

We shall not be held liable for damages caused by an inappropriate use of the device as a personal computer, for instance related to the use of additional software, to network connections, or to a breach of privacy related to the storage of confidential information (such as names of patients) on its hard disk and/or to the use of external storage devices that might be contaminated with spyware.

Quality Control

Each batch is stringently tested for good performance and correctness of results.

List of Components for Separate Order

If required, these reagents for the **ChlamType-23S AS-4 Kit** may be ordered separately:

component	name	category	amount	Cat#	storage
23S_FP	23S_Forward Primer (100 µM)	primer	20 µl	246503501	-20 °C
23S_RP	23S_Reverse Primer_BIOTIN (100 µM)	primer	20 µl		-20 °C
EGFP_FP	EGFP_Forward Primer (10 µM)	primer	20 µl	246503502	-20 °C
EGFP_RP	EGFP_Reverse Primer_BIOTIN (10 µM)	primer	20 µl		-20 °C
C1	Hybridisation Buffer	buffered reagent	30 ml	245105000	18-28 °C
C2	Washing Buffer 1	buffer	120 ml	245106000	18-28 °C
C3	HRP Conjugate 100 x	buffered enzyme	200 µl	245107000	2-8 °C
C4	Conjugate Buffer	buffered reagent	30 ml	245108000	18-28 °C
C5	Washing Buffer 2	buffer	120 ml	245109000	18-28 °C
D1	HRP Substrate	buffered reagent	15 ml	245110000	2-8 °C
ArrayStrips	Chlam AS-4	plugged microarrays	1 strip	240010454	15-28 °C
StripCaps	StripCaps	plastic ware	1 strip	245112000	15-28 °C

For prices please contact us:

Legal Manufacturer

Alere Technologies GmbH
Loebstedter Str. 103-105
07749 Jena, Germany

Contact

If you require any further information on this product please e-mail to cct.home@clondiag.com

LITERATURE

<http://alere-technologies.com/en/products/lab-solutions/chlamydia.html>

UPDATES & SOFTWARE

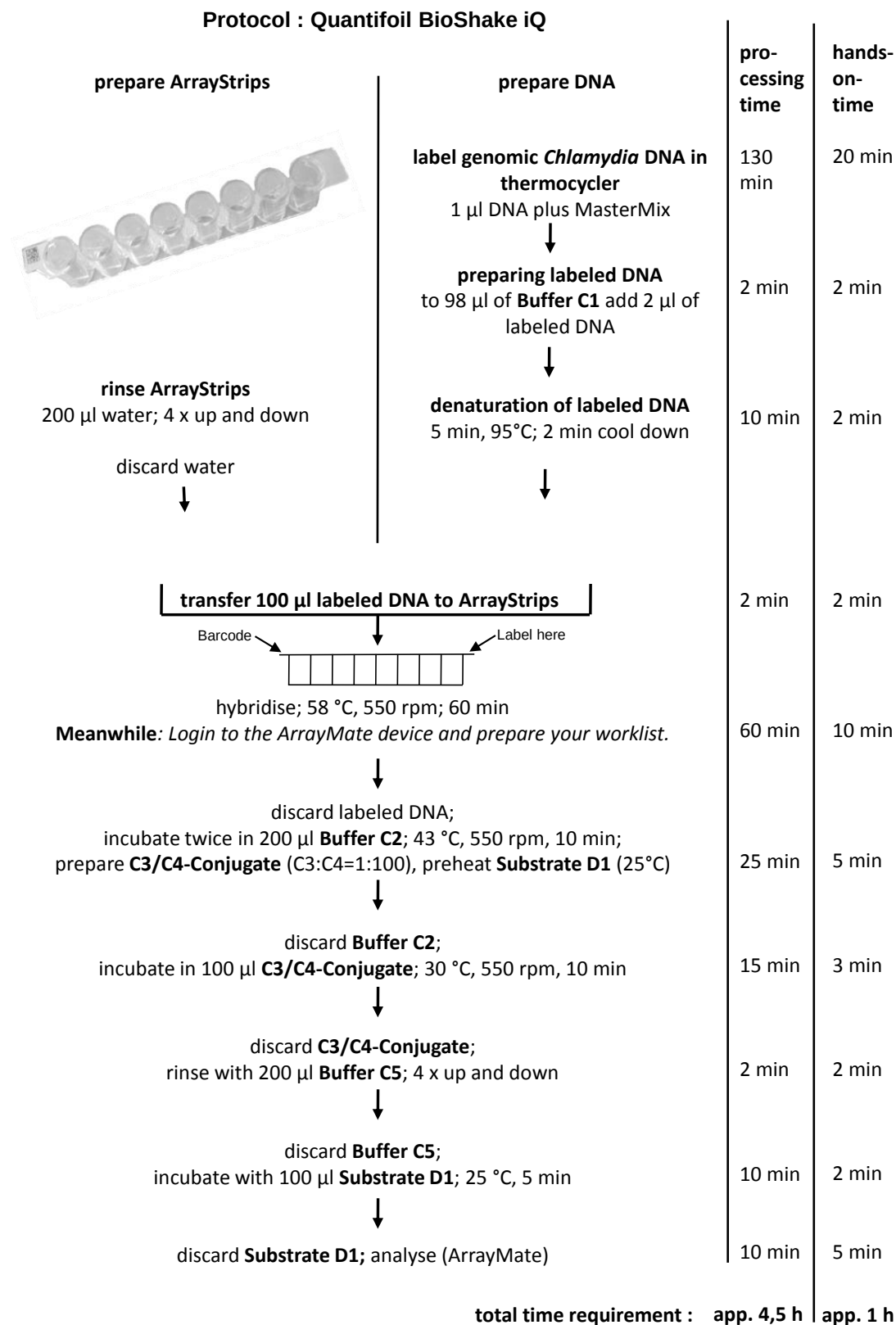
Notifications on database/software updates and freeware tools can be found at

<http://alere-technologies.com/en/science-technologies/publications/downloads.html>.

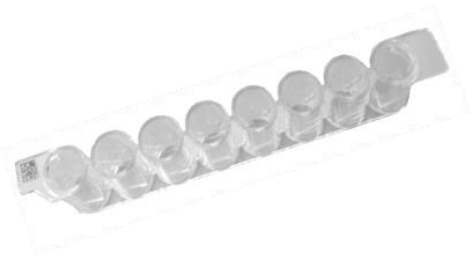
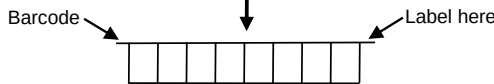
and/or <http://alere-technologies.com/en/news.html>.

APPENDIX 1 – FLOW CHARTS

The figures summarise the test procedure. However, please refer to the text section of this manual at any step of the test protocol for further important details.



Protocol : Eppendorf Thermomixer

Protocol : Eppendorf Thermomixer		pro- cessing time	hands- on- time
prepare ArrayStrips	prepare DNA		
	label genomic <i>Chlamydia</i> DNA in thermocycler 1 µl DNA plus MasterMix	130 min	20 min
rinse ArrayStrips 200 µl water; 4 x up and down	↓ preparing labeled DNA to 98 µl of Buffer C1 add 2 µl of labeled DNA	2 min	2 min
discard water ↓	↓ denaturation of labeled DNA 5 min, 95°C; 2 min cool down	10 min	2 min
↓			
transfer 100 µl labeled DNA to ArrayStrips		2 min	2 min
			
hybridise; 58 °C, 550 rpm; 60 min			
Meanwhile: Login to the ArrayMate device and prepare your worklist.		60 min	10 min
↓ discard labeled DNA; incubate twice in 200 µl Buffer C2 ; 50 °C, 550 rpm, 10 min; prepare C3/C4-Conjugate (C3:C4=1:100), preheat Substrate D1 (25°C)		25 min	5 min
↓ discard Buffer C2 ; incubate in 100 µl C3/C4-Conjugate ; 30 °C, 550 rpm, 10 min		15 min	3 min
↓ discard C3/C4-Conjugate ; rinse with 200 µl Buffer C5 ; 4 x up and down		2 min	2 min
↓ discard Buffer C5 ; incubate with 100 µl Substrate D1 ; 25 °C, 5 min		10 min	2 min
↓ discard Substrate D1 ; analyse (ArrayMate)		10 min	5 min
total time requirement :		app. 4,5 h	app. 1 h

APPENDIX 2 – PROBE TO TARGET TABLE

Target genes	Probes	Function
chlamydia_1	chlamydia_1	genus marker
chlamydophila_1	chlamydophila_1	genus marker
chlamydophila_2	chlamydophila_2	genus marker
pos_56_G	pos_56_G	family marker
pos_56_A	pos_56_A	family marker
pos_56_C	pos_56_C	family marker
pos_56_T	pos_56_T	family marker
C_muridarum	C_mur_1_pm, C_mur_2_mm, Cmur_MoPn_552	genetic marker for C. muridarum
C_suis	C_suis_1, C_suis_2, Csu_H5_549, Csu_S45_1878	genetic marker for C. suis
C_trachomatis	C_trach_1_pm, C_trach_2_mm, C_trach_3_mm, C_mur_2_mm, Ctracho_1885	genetic marker for C. trachomatis
C_abortus	Cabo_B577_533, Cp_abortus_2	genetic marker for C. abortus
C_caviae	Ccav_GPIC_535, Cp_caviae_3, Cp_caviae_4	genetic marker for C. caviae
C_felis	Cfel_Baker_535, Cp_felis_2	genetic marker for C. felis
C_pecorum	Cp_pecorum_1, Cp_pecorum_2, Cpec_IPA_538	genetic marker for C. pecorum
C_pneumoniae	Cp_pneu_1, Cp_pneu_2, Cpneu_TW-183_568_neu, Cpneu_TW-183_siteG	genetic marker for C. pneumoniae
C_psittac	Cp_psittaci_3, Cp_psittaci_4, Cpsi_6BC_1869, Cpsi_WC_535	genetic marker for C. psittac
C_avium	01_C_avium_A1, 04_C_avium_A2, 03_C_avium+gall	genetic marker for C. avium
C_gallinacea	02_C_gallinacea_B1, 05_C_gallinacea_B2, 03_C_avium+gall	genetic marker for C. gallinacea
simkania	simk_negev_1961, S_negev_1	genetic marker for simkania
waddlia	waddlia_chondr_1870, W_chon_1	genetic marker for waddlia
01_Protechlamydiaamoebophila UWE25_00079-00102	01_ProtechlamydiaamoebophilaUWE25_00079-00102	genetic marker for Protechlamydiaamoebophila
02_Protechlamydiaamoebophila UWE25_00093-00119	02_ProtechlamydiaamoebophilaUWE25_00093-00119	genetic marker for Protechlamydiaamoebophila
03_Protechlamydiaaegleriophila Knic_00084-00106	03_ProtechlamydiaaegleriophilaKnic_00084-00106	genetic marker for Protechlamydiaaegleriophila Knic
04_Neochlamydiahartmannellae_00086-00110	04_Neochlamydiahartmannellae_00086-00110	genetic marker for Neochlamydiahartmannellae
05_Parachlamydiaacanthamoebae_00098-00127	05_Parachlamydiaacanthamoebae_00098-00127	genetic marker for Parachlamydiaacanthamoebae
06_Parachlamydiaacanthamoebae_00114-00142	06_Parachlamydiaacanthamoebae_00114-00142	genetic marker for Parachlamydiaacanthamoebae
07_Criblamydiasequanensis_00096-00120	07_Criblamydiasequanensis_00096-00120	genetic marker for Criblamydiasequanensis
08_Criblamydiasequanensis_00123-00146	08_Criblamydiasequanensis_00123-00146	genetic marker for Criblamydiasequanensis
09_ChlamydialesXenoturbella_0102-00132	09_ChlamydialesXenoturbella_00102-00132	genetic marker for ChlamydialesXenoturbella
10_ChlamydialesXenoturbella_0110-00140	10_ChlamydialesXenoturbella_00110-00140	genetic marker for ChlamydialesXenoturbella
16_Estellalalausannensis_00096-00118	16_Estellalalausannensis_00096-00118	genetic marker for Estrellalalausannensis
EGFP	EGFP_745, EGFP_718, EGFP_588	Internal Amplification and Hybridisation Control.
rrl_0101_0177_10:111740	rrl_0101_0177_10:111740	Marker indicating E. coli DNA contamination.

APPENDIX 3 – LIST OF REFERENCE EXPERIMENTS FOR PATTERN MATCH

theoretical reference experiment	Strain affiliation	GenBank ID
C_23s_rt_Chlamydia muridarum AE002280	<i>Chlamydia muridarum</i> AE002280	AE002280.2
C_23s_rt_Chlamydia suis DQ118376	<i>Chlamydia suis</i> DQ118376	DQ118376.1
C_23s_rt_Chlamydia suis U68428	<i>Chlamydia suis</i> U68428	U68428.1
C_23s_rt_Chlamydia suis U73110	<i>Chlamydia suis</i> U73110	U73110.2
C_23s_rt_Chlamydia trachomatis CP000051	<i>Chlamydia trachomatis</i> CP000051	CP000051.1
C_23s_rt_Chlamydia trachomatis U68442	<i>Chlamydia trachomatis</i> U68442	U68442.1
C_23s_rt_Chlamydia trachomatis U68443	<i>Chlamydia trachomatis</i> U68443	U68443.2
C_23s_rt_Chlamydia abortus AY027873	<i>Chlamydia abortus</i> AY027873	AY027873.1
C_23s_rt_Chlamydia abortus AY027874	<i>Chlamydia abortus</i> AY027874	AY027874.1
C_23s_rt_Chlamydia abortus CR848038	<i>Chlamydia abortus</i> CR848038	CR848038.1
C_23s_rt_Chlamydia avium LCL_10071	<i>Chlamydia avium</i> LCL_10071	LCL_10071.1
C_23s_rt_Chlamydia caviae AE016997	<i>Chlamydia caviae</i> AE016997	AE016997.1
C_23s_rt_Chlamydia felis U68458	<i>Chlamydia felis</i> U68458	U68458.1
C_23s_rt_Chlamydia felis U68459	<i>Chlamydia felis</i> U68459	U68459.1
C_23s_rt_Chlamydia gallinacea AWUS01000004	<i>Chlamydia gallinacea</i> AWUS01000004	AWUS01000004.1
C_23s_rt_Chlamydia ibidis APJW01000003	<i>Chlamydia ibidis</i> APJW01000003	APJW01000003.1
C_23s_rt_Chlamydia pecorum U68434	<i>Chlamydia pecorum</i> U68434	U68434.4
C_23s_rt_Chlamydia pneumoniae AE017160	<i>Chlamydia pneumoniae</i> AE017160	AE017160.1
C_23s_rt_Chlamydia pneumoniae BA000008	<i>Chlamydia pneumoniae</i> BA000008	BA000008.3
C_23s_rt_Chlamydia pneumoniae U68423	<i>Chlamydia pneumoniae</i> U68423	U68423.1
C_23s_rt_Chlamydia pneumoniae U68425	<i>Chlamydia pneumoniae</i> U68425	U68425.1
C_23s_rt_Chlamydia pneumoniae U68426	<i>Chlamydia pneumoniae</i> U68426	U68426.2
C_23s_rt_Chlamydia psittaci AF481048	<i>Chlamydia psittaci</i> AF481048	AF481048.1
C_23s_rt_Chlamydia psittaci U68419	<i>Chlamydia psittaci</i> U68419	U68419.2
C_23s_rt_Chlamydia psittaci U68447	<i>Chlamydia psittaci</i> U68447	U68447.2
C_23s_rt_Chlamydia psittaci U68449	<i>Chlamydia psittaci</i> U68449	U68449.1
C_23s_rt_Simkania negevensis U68460	<i>Simkania negevensis</i> U68460	U68460.2
C_23s_rt_Waddlia chondrophila AF346001	<i>Waddlia chondrophila</i> AF346001	AF346001.1
C_23s_rt_Waddlia sp. G817 AY184804	<i>Waddlia</i> sp. G817 AY184804	AY184804.1
C_23s_rt_Parachlamydia acanthamoebae Y07555	<i>Parachlamydia acanthamoebae</i> Y07555	Y07555.1
C_23s_rt_Candidatus Protochlamydia amoebophila BX908798	<i>Candidatus Protochlamydia amoebophila</i> BX908798	BX908798.1
C_23s_rt_neg ctrl	negative control	

practical reference experiment	Strain affiliation
C_23s_rp_C.abortus S26/3 (DC59)	<i>Chlamydia abortus</i> S26/3 (DC59)
C_23s_rp_C.avium DC88	<i>Chlamydia avium</i> DC88
C_23s_rp_C.caviae DC25	<i>Chlamydia caviae</i> DC25
C_23s_rp_C.felis DC26	<i>Chlamydia felis</i> DC26
C_23s_rp_C.gallinacea_DC63	<i>Chlamydia gallinacea</i> _DC63
C_23s_rp_C.pecorum DC50	<i>Chlamydia pecorum</i> DC50
C_23s_rp_C.pneumoniae DC40	<i>Chlamydia pneumoniae</i> DC40
C_23s_rp_C.psittaci 6BC(DC45)	<i>Chlamydia psittaci</i> 6BC(DC45)
C_23s_rp_C.muridarum DC39	<i>Chlamydia muridarum</i> DC39
C_23s_rp_C.suis DC19	<i>Chlamydia suis</i> DC19
C_23s_rp_C.suis field sample	<i>Chlamydia suis</i> field sample
C_23s_rp_C.trachomatis DC38	<i>Chlamydia trachomatis</i> DC38
C_23s_rp_Simkania DC48	<i>Simkania</i> DC48
C_23s_rp_Waddlia chondrophila_CD17	<i>Waddlia chondrophila</i> _CD17

APPENDIX 4 – HTML REPORT OF THE REFERENCE ISOLATE DC.38 TRACHOMATIS

Page 1

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Operator	
Sample ID	03-F.273884110454_6_C.trachomatis DC38
Experiment ID	03-F.273884110454_6_C.trachomatis DC38
Date of Result	Tue Sep 16 08:47:24 2014
Assay Name	Chlamydia_4
Assay Type	23s
Assay ID	10454
Well Position	---
Software Version	2014-09-12
Device	---

Best 3 Assignments after Pattern Match with Reference Experiments

strain	score	explanation
C_23s_rp_C.trachomatis DC38	0.579222	The lowest score indicates the best match.
C_23s_rt_neg ctrl	5.541616	
C_23s_rp_C.muridarum DC39	5.756433	

Controls

control	result	explanation
staining control	passed	"Failed" indicates possible problems originating from the hybridization or the staining procedure.
negative control	passed	"Failed" indicates possible problems originating from the staining procedure.

Internal Amplification and Hybridisation Control

control	result	explanation
EGFP_588	positive	At least one of three EGFP probes have to be positive, if you use the Internal Amplification and Hybridisation Control.
EGFP_718	positive	
EGFP_745	positive	
rrl_0101_0177_10	negative	Marker indicating <i>E. coli</i> DNA contamination.

Family

affiliation	classification	explanation
pos_56_A	positive	positive with <i>C. trachomatis</i> , <i>C. suis</i> , <i>C. caviae</i> or <i>C. pneumoniae</i> samples
pos_56_C	negative	positive with <i>C. abortus</i> , <i>C. psittaci</i> , <i>C. felis</i> , <i>C. avium</i> or <i>C. gallinacea</i> samples

pos_56_G	negative	positive with C. muridarum samples
pos_56_T	negative	positive with C. pecorum or C. ibidis samples
Genus		
marker	classification	explanation
chlamydia_1	positive	positive with C. trachomatis, C. suis or C. muridarum samples
chlamydophila_1	negative	positive with C. abortus, C. psittaci, C. felis, C. avium, C. gallinacea, C. caviae, C. pneumonia, C. ibidis or C. pecorum samples
chlamydophila_2	negative	positive with C. abortus, C. psittaci, C. felis, C. avium, C. gallinacea, C. caviae, C. pneumonia, C. ibidis or C. pecorum samples
Chlamydia Species Assignment after Signal Interpretation		
marker	classification	explanation
C. abortus	negative	
C. caviae	negative	
C. felis	negative	
C. psittaci	negative	
C. pecorum	negative	
C. pneumoniae	negative	
C. suis	negative	
C. muridarum	negative	
C. trachomatis	positive	
C. avium	negative	
C. gallinacea	negative	
Other Chlamydiales		
marker	classification	explanation
Simkania	negative	
Waddlia	negative	
01 Protochlamydia amoebophila	negative	
02 Protochlamydia amoebophila	negative	
Protochlamydia naegleriophila Knic	negative	
Neochlamydia hartmannellae	negative	
01 Parachlamydia acanthamoebae	negative	
02 Parachlamydia acanthamoebae	negative	
01 Criblamydia sequanensis	negative	
02 Criblamydia sequanensis	negative	
01 Chlamydiales	negative	

Xenoturbella		
02 Chlamydiales Xenoturbella	negative	
Estrella lausannensis	negative	

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Operator

Sample ID 03-F.273884110454_6_C.trachomatis DC38

Experiment ID 03-F.273884110454_6_C.trachomatis DC38

Date of Result Tue Sep 16 08:47:24 2014

Assay Name Chlamydia_4

Assay Type 23s

Assay ID 10454

Well Position ---

Software Version 2014-09-12

Device ---

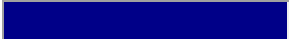
Controls

marker	value	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0,1M NaPP Standard pH 9	0.002										
biotin	0.607										
rrl_0101_0177_10.	0.001										
EGFP_745.	0.394										
EGFP_718.	0.679										
EGFP_588.	0.664										

Family

marker	value	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
pos_56_A.	0.245										
pos_56_C.	0.004										
pos_56_G.	0.002										
pos_56_T.	0.003										

Genus

marker	value	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
chlamydia_1.	0.518										
chlamydophila_1.	0.045										

chlamydomphila_2.	0.006	
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Chlamydia Species

marker	value	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Cp_abortus_2	0.0										
Cabo_B577_533	0.001										
01_C_avium_A1	0.001										
04_C_avium_A2	0.121										
03_C_avium+gall	0.729										
Cp_caviae_3	-0.001										
Cp_caviae_4	0.005										
Ccav_GPIC_535	0.001										
Cp_felis_2	0.0										
Cfel_Baker_535	0.003										
02_C_gallinacea_B1	0.002										
05_C_gallinacea_B2	0.291										
C_mur_1_pm	0.02										
C_mur_2_mm	0.593										
Cmur_MoPn_552	-0.002										
Cp_pecorum_1	-0.001										
Cp_pecorum_2	0.002										
Cpec_IPA_538	0.0										
Cp_pneu_1	0.002										
Cp_pneu_2	0.0										
Cpneu_TW-183_568_neu	0.001										
Cpneu_TW-183_siteG	-0.002										
Cp_psittaci_3	0.006										
Cp_psittaci_4	0.002										
Cpsi_6BC_1869	0.001										
Cpsi_WC_535	0.002										
C_suis_1	0.0										
C_suis_2	0.013										
Csu_H5_549	0.002										
Csu_S45_1878	0.004										
C_trach_1_pm	0.667										
C_trach_2_mm	0.641										
C_trach_3_mm	0.47										
Ctracho_1885	0.483										

Other Chlamydiales

marker	value	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
S_negev_1	-0.002										
simk_negev_1961	-0.003										
W_chon_1	-0.002										
waddlia_chondr_1870	0.0										
01 Protochlamydia amoebophila.	-0.001										
02 Protochlamydia amoebophila.	0.002										
Protochlamydia naegleriophila Knic.	-0.003										
Neochlamydia hartmannellae.	-0.001										
01 Parachlamydia acanthamoebae.	0.004										
02 Parachlamydia acanthamoebae.	0.003										
01 Criblamydia sequanensis.	-0.001										
02 Criblamydia sequanensis.	0.001										
01 Chlamydiales Xenoturbella.	0.003										
02 Chlamydiales Xenoturbella.	0.001										
Estrella lausannensis.	0.002										